CellEvent™ Caspase-3/7 Detection Reagents (Green/Red)

Catalog Numbers C10423, C10723, C10432, C10433, C10430, and C10431

Pub. No. MAN0028521 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent and CellEvent™ Caspase-3/7 Red Detection Reagent are novel fluorogenic substrates that enable detection of activated caspase-3 and caspase-7 proteins in apoptotic cells. The cell-permeant reagents consist of a four-amino acid peptide (DEVD) that is conjugated to a nucleic acid-binding dye. In non-apoptotic cells, the reagents are non-fluorescent. However, during apoptosis, activated caspase-3 and caspase-7 cleave the DEVD peptide, enabling the dye to bind to DNA and produce a bright, fluorogenic response (see "Spectral properties" on page 4). Because the cleaved reagent labels nuclei of caspase-3/7-positive cells, this stain can also provide information on nuclear morphology, including condensed nuclei that are typical of late-stage apoptosis.

Key features of the reagents include the following:

- **Streamlined protocol**—Add the reagent to cells, incubate for 30–60 minutes, then measure fluorescence; no cell lysis or wash steps required (see Figure 1 and "Perform an endpoint assay" on page 3).
- **Optimized for apoptosis analysis**—Highly specific for caspase-3/7 activation (see Figure 4).
- **Flexible imaging options**—Compatible with traditional and live-cell fluorescence microscopy, and formaldehyde-based fixation methods.
- **Multiplex-enabled**—Combine with other fluorescent reagents to detect other proteins of interest or to confirm apoptosis in the same cell or cell population (see Figure 6).
- **Flexible workflows**—Excellent Z-factor (Z') value indicates suitability for high-content analysis (HCA), high-throughput screening (HTS), and fluorescence microplate readers.

This guide describes how to perform an endpoint or kinetic (dynamic measurement) apoptosis assay using CellEvent™ Caspase-3/7 Detection Reagents.

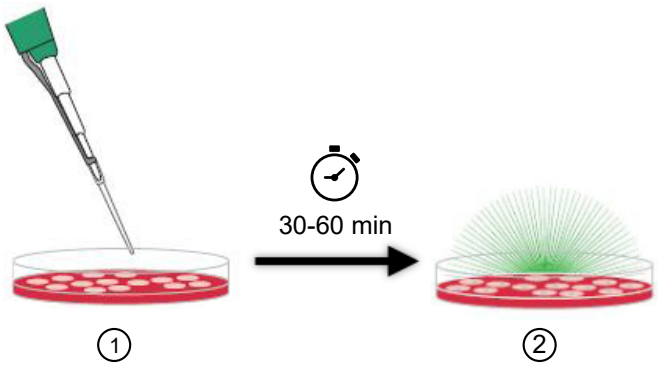

Figure 1 Endpoint assay workflow using the CellEvent™ Caspase-3/7 Green Detection Reagent

① Add diluted CellEvent™ Caspase-3/7 Green Detection Reagent to cells
② Measure fluorescence

Contents and storage

Table 1 CellEvent™ Caspase-3/7 Detection Reagent (green/red)

Cat. No.	Amount	Form	Excitation/Emission	Storage[1]
CellEvent™ Caspase-3/7 Green Detection Reagent				
C10723	25 µL	2 mM in DMSO (400X)	502/530 nm	–5 to –30°C Protect from light.
C10423	100 µL			
C10432	1 vial	Dry down, powder		
C10433	5 vials			
CellEvent™ Caspase-3/7 Green ReadyProbes™ Reagent				
R37111	1.75 mL in dropper bottle	12.5X in PBS (Ready-to-use)	502/530 nm	–5 to –30°C Protect from light.
CellEvent™ Caspase-3/7 Red Detection Reagent				
C10430	1 vial	Dry down, powder	590/610 nm	–5 to –30°C Protect from light.
C10431	5 vials			

[1] When stored as directed, the product is stable for at least 6 months from date of receipt.

Table 2 CellEvent™ Caspase Variety Pack (Cat. No. C10434)

Item	Amount	Storage [1]
CellEvent™ Caspase-3/7 Green Detection Reagent (dry down, powder)	1 vial	–5 to –30°C Protect from light.
CellEvent™ Caspase-3/7 Red Detection Reagent (dry down, powder)	1 vial	

[1] When stored as directed, the product is stable for at least 6 months from date of receipt.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Fetal bovine serum, certified, heat inactivated, US origin	10082147
DPBS, calcium, magnesium (or equivalent)	14040133
(Optional) Complete medium[1]	MLS
(Optional) Fixative (e.g., 3.7% formaldehyde in PBS)	MLS
(Optional) ProLong™ Glass Antifade Mountant or SlowFade™ Glass Antifade Mountant	P36980 S36917

[1] For use as a diluent for the CellEvent™ Caspase-3/7 detection reagent.

Procedural guidelines

- The following staining procedure was optimized using HeLa, A673, and U-2 OS cells that were stained with CellEvent™ Caspase-3/7 Detection Reagent (green/red) at the recommended concentrations of 5 µM (green) and 30 µM (red). If needed, the procedure can be adapted for use with other cell types.
- Growth medium, cell density, cell type variations, and other factors can influence staining.
- In initial experiments, we recommend testing a range of concentrations for the CellEvent™ Caspase-3/7 Detection Reagent to determine the optimal conditions for your model.

Before you begin

Prepare fresh staining solution of CellEvent™ Caspase-3/7 Detection Reagent (green or red) on each day of use.

Note: We recommend that you prepare a 10X staining solution, then add directly to cells growing in cell-culture medium. For example, if you are performing the assay in a 96-well plate, you will add 10 µL of 10X staining solution to each well containing 90 µL of cell-culture medium.

Reagent	Action
CellEvent™ Caspase-3/7 Green ReadyProbes™ Reagent (Cat. No. R37111)	Add two drops (40 µL each drop) to 1 mL of complete cell-culture medium.
CellEvent™ Caspase-3/7 Green Detection Reagent (2 mM in DMSO; Cat. No. C10723, C10423)	To prepare a 10X staining solution, dilute 1:40 with complete cell-culture medium (or other desired vehicle). When added to cells at a 1:10 dilution, the final concentration will be 5 µM. **Note:** The final concentration can be optimized between 2–10 µM for other cell types.
CellEvent™ Caspase-3/7 Green Detection Reagent (dry down, powder; Cat. No. C10432, C10433) CellEvent™ Caspase-3/7 Red Detection Reagent (dry down, powder; Cat. No. C10430, C10431)	1. Briefly centrifuge the vial containing the dry powder to collect the contents. 2. Prepare a 100X stock solution—Add 100 µL of PBS (or equivalent) to the vial, then vortex thoroughly to ensure complete dissolution of the powder. 3. Centrifuge the 100X stock solution to collect the contents. **Note:** The 100X stock solution can be stored frozen for 1 month or at 2–4°C for 1 week. Avoid multiple freeze-thaw cycles. 4. To prepare a 10X staining solution, dilute the 100X stock solution 1:10 with complete cell-culture medium (or other desired vehicle). **Note:** It is normal for CellEvent™ Caspase-3/7 Red Detection Reagent to impart a light purple color to cell-culture media, depending on the pH. Ensure that the reagent is thoroughly mixed with the cell sample at the time of staining.

Perform an endpoint assay

1. Prepare the appropriate apoptotic inducer, such as 5-µM camptothecin, then treat cells for the desired amount of time.

   Note: We recommend that you prepare fresh apoptotic inducer on each day of use for optimal caspase activation.

2. Prepare a 10X staining solution of CellEvent™ Caspase-3/7 Detection Reagent (green or red) in complete medium or other desired vehicle (see "Before you begin" on page 3).

3. Add the 10X staining solution directly to the cells at a 1:10 dilution.

   For example, if you are performing the assay in a 96-well plate, add 10 µL of 10X staining solution to each well containing 90 µL of cell-culture medium.

4. Incubate for 30–60 minutes at 37°C.

5. *(Optional)* Preserve the cells with a formaldehyde-based fixative. We recommend using 3.7% formaldehyde for 15 minutes, but this can be adjusted according to the cell type.

6. *(Optional)* Stain the cells with a nuclear stain or counter stain.

7. *(Optional)* Mount the cells to stabilize and prolong the signal. We recommend using one of the following mountants.

   - For hard-cure, overnight mounting—Use ProLong™ Glass Antifade Mountant (Cat. No. P36980).
   - For soft-cure, quick mounting—Use SlowFade™ Glass Antifade Mountant (Cat. No. S36917).

8. Image the cells using the appropriate instrument filter set or laser configuration (see "Spectral properties" on page 4).

Reagent	Recommended filter set
CellEvent™ Caspase-3/7 Green Detection Reagent	FITC/GFP
CellEvent™ Caspase-3/7 Red Detection Reagent	Texas Red

Perform a kinetic assay

1. Prepare a 10X staining solution of CellEvent™ Caspase-3/7 Detection Reagent (green or red) in complete medium or other desired vehicle (see "Before you begin" on page 3).

2. Add the 10X staining solution directly to the cells at a 1:10 dilution.

   For example, if you are performing the assay in a 96-well plate, add 10 µL of 10X staining solution to each well containing 90 µL of cell-culture medium.

3. Add the appropriate apoptotic inducer, such as 5-µM camptothecin.

   Note: We recommend that you prepare fresh apoptotic inducer on each day of use for optimal caspase activation.

4. Return the cells to an incubator set at 37°C.

5. At desired time points, remove the cells from the incubator, then image the cells using the appropriate instrument filter set or laser configuration (see "Spectral properties" on page 4).

Reagent	Recommended filter set
CellEvent™ Caspase-3/7 Green Detection Reagent	FITC/GFP
CellEvent™ Caspase-3/7 Red Detection Reagent	Texas Red

Note: For dynamic cell monitoring or capturing apoptosis progression in time lapse videos, we recommend using the EVOS™ M7000 Imaging System (Cat. No. AMF7000), equipped with the EVOS™ Onstage Incubator (Cat. No. AMC1000).

Spectral properties

- CellEvent™ Caspase-3/7 Green Detection Reagent absorbs and emits optimally at 502 nm and 530 nm, respectively (see Figure 2). Fluorescence can be visualized with a standard FITC or GFP filter.

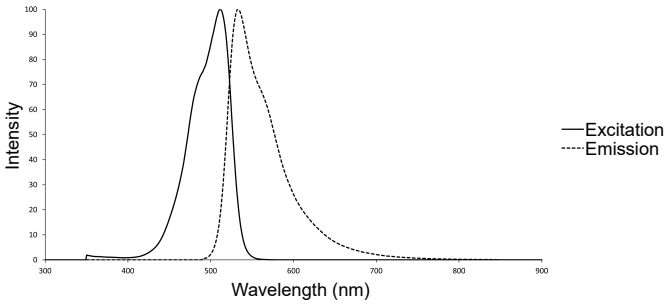

Figure 2 CellEvent™ Caspase-3/7 Green Detection Reagent: Fluorescence excitation and emission spectra after caspase-3/7 activation

- CellEvent™ Caspase-3/7 Red Detection Reagent absorbs and emits optimally at 590 nm and 610 nm, respectively (see Figure 3). Fluorescence can be visualized with a Texas Red filter.

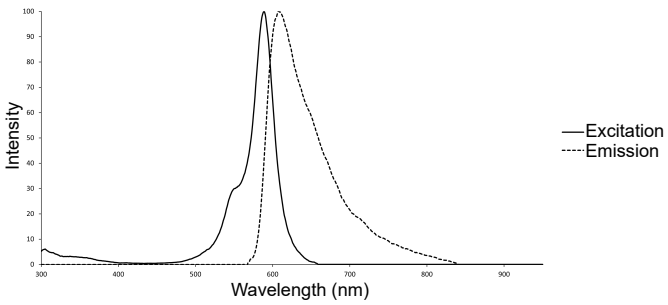

Figure 3 CellEvent™ Caspase-3/7 Red Detection Reagent: Fluorescence excitation and emission spectra after caspase-3/7 activation

Performance characteristics

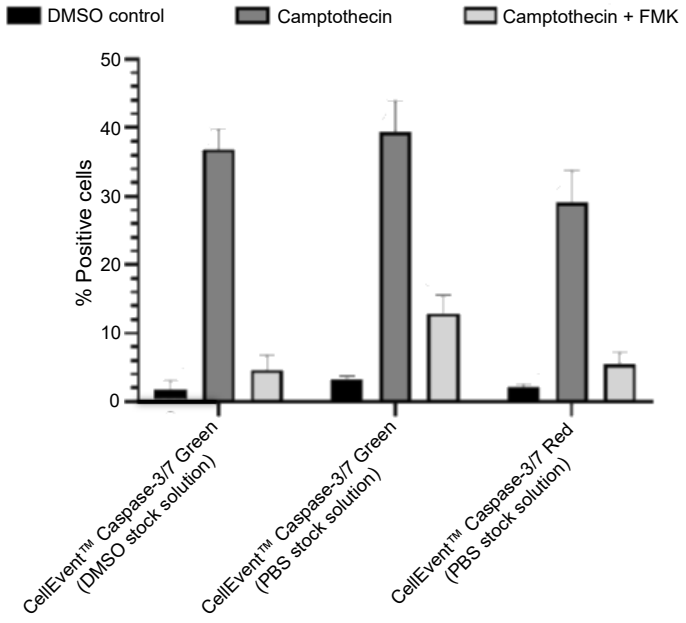

Figure 4 Specificity of CellEvent™ Caspase-3/7 Detection Reagents (green/red)

A673 cells were seeded at 2,000 cells per well, then treated with DMSO/PBS control, 2-µM camptothecin, or 2-µM camptothecin plus 10-µM caspase-3/7 inhibitor (FMK) for 24 hours at 37°C. Cells were then stained with CellEvent™ Caspase-3/7 Green Detection Reagent from a DMSO stock solution, or lyophilized CellEvent™ Caspase-3/7 Detection Reagent (green or red) formulated in PBS. Following a 1-hour incubation at 37°C, cells were analyzed using the CellInsight™ CX5 High Content Screening Platform.

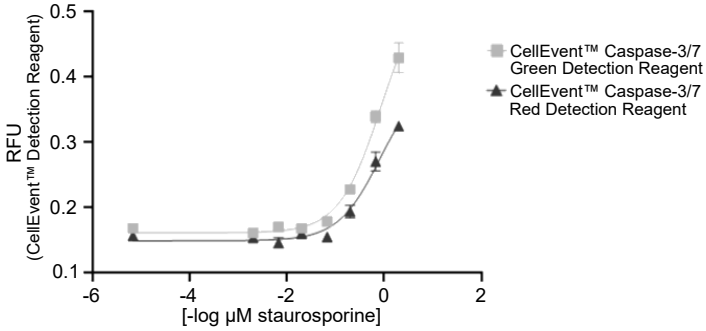

Figure 5 Dose response curve of CellEvent™ Caspase-3/7 Detection Reagents (green/red)

A673 cells were seeded at 5,000 cells per well, then treated with staurosporine at the indicated concentration for five hours at 37°C. Following induction of apoptosis, cells were stained with lyophilized CellEvent™ Caspase-3/7 Detection Reagent (green or red) formulated in PBS for 1 hour at 37°C. Cells were analyzed using the Varioskan™ LUX microplate reader.

Typical results

Apoptotic cells with activated caspase-3/7 show bright green or red nuclei, while cells without activated caspase-3/7 exhibit minimal fluorescence signal (see Figure 6).

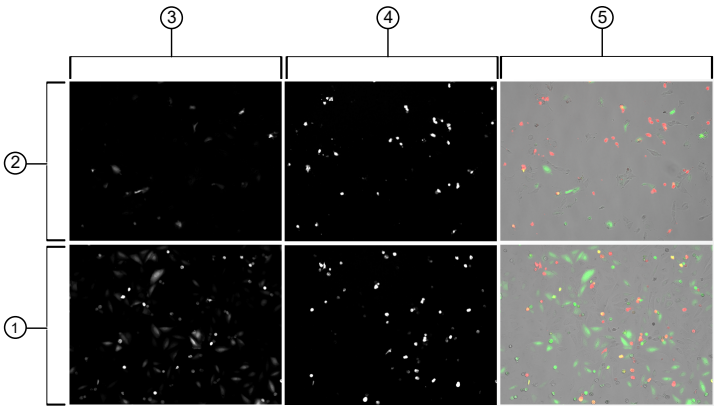

Figure 6 GFP compatibility with CellEvent™ Caspase-3/7 Red Detection Reagent signal

A673 and U-2 OS cells were plated at 3,000 cells per well, then transduced with cytosolic GFP BacMam reagent. After treatment with 2-µM camptothecin for 24 hours at 37°C, cells were stained with CellEvent™ Caspase-3/7 Red Detection Reagent for 1 hour at 37°C, then imaged on the EVOS™ M7000 Imaging System. The CellEvent™ Caspase-3/7 Red Detection Reagent signal can be detected in cells expressing cytosolic GFP with no discernible crosstalk between Texas Red and GFP channels.

① A673 cells
② U-2 OS cells
③ GFP
④ Texas Red
⑤ GFP and Texas Red

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
CellROX™ Deep Red Reagent, for oxidative stress detection	C10422
Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL solution in water	H3570
Tetramethylrhodamine, Methyl Ester, Perchlorate (TMRM)	T668
LC3B Antibody Kit for Autophagy	L10382
ProLong™ Glass Antifade Mountant	P36980
SlowFade™ Glass Antifade Mountant	S36917
DPBS, calcium, magnesium	14040133
Fetal bovine serum, certified, heat inactivated, US origin	10082147

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 29851 Willow Creek Road | Eugene, Oregon 97402 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0028521

Revision	Date	Description
A.0	14 November 2022	New document for CellEvent™ Caspase-3/7 detection reagents (green/red).